RESEARCH PAPER | Microbiology | Volume : 4 | Issue : 3 | Mar 2014 | ISSN - 2249-555X

Detection of Chromobacterium Violaceum in Pasteurized Milk Samples From an Organized Dairy Plant in Thrissur, Kerala

KEYWORDS: Chromobacterium violaceum, pasteurized milk, antibiogram

Ambily R	Beena A.K.	Krishna, S. V.
Assistant Professor, Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy	Assistant Professor, College of Dairy Science and Technology, Mannuthy	Assistant Professor, College of Veterinary Science, Karim Nagar, AP

ABSTRACT *This report includes the identification and antibiogram study of Chromobacterium violaceum obtained from pasteurized milk sample collected from an organized dairy plant in Thrissur district of Kerala. The deep violet colored colonies obtained on the nutrient agar plate during the bacteriological analysis of the pasteurized milk samples were subjected to staining and biochemical characterization, the results of which indicated the identity of isolate as Chromobacterium violaceum. In the attempt made to trace the source of this organism, C. violaceum was isolated from the water samples collected from the dairy plant. The antibiogram revealed that the organism was resistant to cephalosporins and penicillin group of antibiotics. The total viable count, coliform count and E. coli count of the milk samples were well within the limits prescribed by FSSA. The presence of an opportunistic pathogen like C. violaceum in processed milk samples which conforms to microbiological standards warrants further investigation.*

Introduction

Chromobacterium violaceum is considered as an opportunistic pathogen of extreme virulence (Yang and Li, 2011). The infection has immense public health impact due to its high mortality rate in human beings (Rai et al., 2011) and animals (Liu et al., 2012). The organism belongs to the Family Neisseriacea of β-Proteobacteria. It is a Gram negative, flagellated heterotroph which lives in a variety of ecosystems in tropical and subtropical regions, including the soil and water. The colonies can be identified on conventional culture media by its striking deep purple pigment (Mahmud et al., 2009). This pigmentation which is unique to this bacteria is due to the production of a chemical called violacein (August et al., 2000), a natural antibiotic useful in the treatment of cancer. *C. violaceum* was first identified as a human pathogen in Malaysia in 1927 (Rai et al., 2011). There are reports of clinical infection (Karthik et al., 2012) and environmental isolation (Narayanan et al., 2012) of *C. violaceum* from different parts of India including the state of Kerala. This is a report of detection of *C. violaceum* from pasteurized milk and water samples from an organized dairy plant.

Materials and Methods

Pasteurized milk sample from an organized dairy plant in Thrissur district of Kerala was analyzed for its bacteriological quality. The milk samples were collected aseptically over a period of two months (June - July) at weekly intervals. The microbiological quality was assessed in terms of total viable count (TVC), coliform count and *E. coli* count in Nutrient agar, Violet Red Bile agar and Eosin Methylene Blue agar respectively as per the standard procedures (Harrigan, 1998). The typical dark violet colored colonies obtained on TVC nutrient agar plates were subjected to biochemical characterization (Barrow and Feltham, 1993).

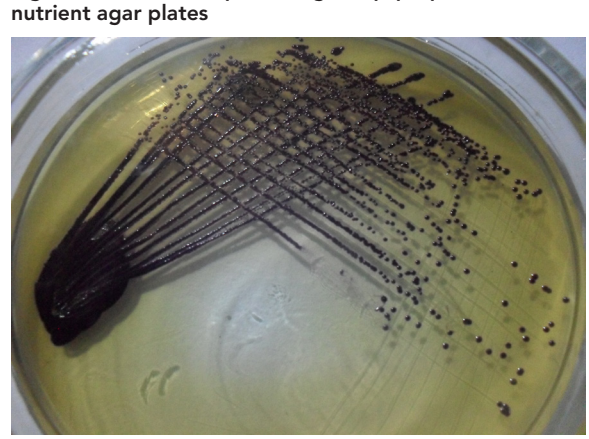
To trace the source of this organism, water samples were collected from five pre determined sites of the dairy plant in the immediate week of detecting violet coloured colonies from milk sample. The work was carried out in the Department of Dairy Microbiology, College of Dairy Science and Technology, Mannuthy, Thrissur. The purplish colonies obtained on nutrient agar from the water samples were also identified. The antibiotic susceptibility test was performed for the isolate from pasteurized milk in Mueller-Hinton agar using the Kirby-Bauer disc diffusion method (Bauer et al., 1966).

Results and discussion

The present study was conducted to assess the microbiological quality of pasteurized milk in an organized dairy plant in Thrissur. The TVC ranged from $1x10^2$ to $1x\ 10^3$ cfu / ml. Coliform count was less than 10/g in all the samples and *E. coli* was not detected in any of the samples. As per FSSAI (2011), counts were well within the limits. While taking TVC, deep purple, mucoid, shiny, low convex, smooth, non gelatinous colonies were detected on nutrient agar in three consecutive samples (Fig. 1). Biochemical characterization of the colonies was suggestive of the isolate to be *C. violaceum* (Table 1).

There are reports of isolation of *C. violaceum* from raw milk, separated milk and separator drain in organized dairy plant (Reid, 1997). However they could not detect the organism in pasteurized milk. Contrary to this report, in this study, *C. violaceum* was identified in pasteurized milk samples. One of the water samples collected from the dairy plant was found to be contaminated with the same bacteria indicating that the water used in the plant could be a potent source. This organism is reported to be an inhabitant of soil and water (Mahmud et al., 2009).

Fig. 1. C. violaceum producing deep purple colonies on nutrient agar plates



Volume : 4 | Issue : 3 | Mar 2014 | ISSN - 2249-555X

Table 1. Biochemical characterization of the isolate

Test	Result
Gram staining	Negative
Shape	bacilli
motility	+
oxidation /fermentation	+/+
catalase	+
oxidase	+
nitrate	+
esculin	-
urease	-
indole	-
Methyl Red test	-
Voges Proskauer test	–
citrate	+
Lysine decarboxylation	-
Ornithine decarboxylation	+
Arginine decarboxylation	+
Glucose	+ (without gas production)
Sucrose	-
Lactose	-
Arabinose	-
Maltose	-
Mannitol	-
Xylose	-
Mannose	+
Trehalose	+
Fructose	+

A salient observation in this study is that prevalence of this organism was noticed in the rainy season. Seasonal occurrence has been reported by Bosch et al. (2008). However, there were no reports of infection during the study period in the area.

Although found in soil and water of tropical areas, infection in humans is rare, but has a high mortality when it occurs (Manjunath, 2007). The identification of virulence genes in the *C. violaceum* genome and secreted virulence factors indicate that the bacterium does have a genetic potential for being pathogenic (Ciprandi et al., 2013 and Gomes et al., 2014). The infection is reported to be severe in immunocompromised and malnourished individuals (Rashid et al., 2013). The symptoms include sepsis and abscesses in multiple organs such as the liver (Orsetti et al., 2013), skin, lungs, lymph nodes and brain (Chen et al., 2003). The waterborne infections causing diarrhoea have been a cause of concern in under developed countries, but an infection with symptoms such as sepsis and abscesses in multiple organs might not be easily diagnosed or even traced to water. The infection occurs as a short-duration, highly virulent disease; therefore, the need for a rapid diagnosis and antibiotic susceptibility profile is urgent (Campbell et al., 2013).

The phenomenon of intrinsic antimicrobial resistance in *C. violaceum* is well documented (Fantinatti-Garboggini et al., 2004). It was found to be resistant to a relatively broad range of antibiotics, a phenomenon that makes the treatment of infections difficult (Rai et al., 2011). In the present study, the antibiogram showed that the isolate was resistant to ampicillin, penicillin, cephalexin, cefuroxime, ceftriaxone, ceftazidime and susceptible to gentamicin, chloramphenicol, ciprofloxacin, co-trimoxazole. The results obtained with the antimicrobial sensitivity test were very similar to those observed in other studies (Dutta and Dutta, 2003). Although there are several extensively occurring waterborne pathogens that is to be considered with greater concern, factors like high virulence and antibiotic resistance, indicates the necessity of further investigation into the presence of *C. violaceum* in processed milk and untreated potable water. In the existing circumstances, monitoring of the water sources and the necessary interventions can minimize the entry of this potentially virulent pathogen into the food chain.

Conclusion

As there was increasing number of reports of clinical incidence and environmental occurrence of *C. violaceum* in the state of Kerala, a long term monitoring programme is recommended to investigate the epidemiology and ecology of the organism.

REFERENCE

1. August, P. R., Grossman, T. H., Minor, C., Draper, M. P., MacNeil, I. A., Pemberton, J. M., Call, K. M., Holt, D., Osbourne, M. S. (2000). Sequence analysis and functional characterization of the violacein biosynthetic pathway from Chromobacterium violaceum. Journal of Molecular Microbiology and Biotechnology, 2(4), 513-519. | 2. Barrow, C. I., & Feltham, R.K.A. (1993). Cowan and Steels Manual for the identification of Medical bacteria (3rd ed.). Cambridge University Press, Great Britain. | 3. Bauer, A. W., Kirby, W. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45(4), 493-496. | 4. Bosch, F. J., Badenhorst, L., Le Roux, J. A., & Louwdoi V. J. (2008). Successful treatment of Chromobacterium violaceum sepsis in South Africa. Journal of Medical Microbiology, 57 (10), 1293-1295. | 5. Campbell, J. I., Lan, N. P. H., Qui, P. T., Dung, L. T., Farrar, J. J. & Baker, S. (2013). A successful antimicrobial regime for Chromobacterium violaceum induced bacteremia. BMC Infectious Diseases, 13, 4. doi:10.1186/1471-2334-13-4 | 6. Chen, C. H., Lin, L. C., Liu, C. E. & Young, T. C. (2003). Chromobacterium violaceum infection: a case report. Journal of Microbiology Immunology and Infection, 36(2), 141–144. | 7. Ciprandi, A., da Silva, W. M., Santos, A. V., de Castro Pimenta, A. M., Carepo, M. S. P., | Schneider, M. P. C., Azevedo, V., & Silva, A. (2013). Chromobacterium violaceum: Important insights for virulence and biotechnological potential by exoproteomic studies. Current Microbiology, 67,100–106. doi 10.1007/s00284-013-0334-5 | 8. Dutta, S., & Dutta, S. K. (2003). Multidrug resistant Chromobacterium violaceum: An unusual bacterium causing long standing wound abscess. Indian Journal of Medical Microbiology, 21, 217-218 | 9. Fantinatti-Garboggini, F., Almeida, R., Portillo, V. A., Barbosa, T. A., Trevilato, P. B., Neto, C. E., Coelho, R. D., Silva, D. W., Bartoleti, L. A., Hanna, E. S., Brocchi, M., Manfio, G. P. (2004). Drug resistance in Chromobacterium violaceum. Genetics and Molecular Research, 3, 134-147. | 10. Food safety and standards Authority of India. (2011). Food safety and Standards (Food product standards and food additives) regulation, part II: 497. | 11. Gomes, T. C., Cardoso, M. S., DaRocha, Laibida, L. A., Nascimento, A. M. A., Zuccherato, L. W., Horta, M. F., Bemquerer, M. P., & Teixeira, S. M. R. (2014) Identification of secreted virulence factors of Chromobacterium violaceum. The Journal of Microbiology, doi:10.1007/s12275-014-3202-5 | 12. Harrigan, W. F. (1998). Laboratory methods in food microbiology. 3rd edition. Academic Press, U.K. | 13. Karthik, R., Pancharatnam, P., & Balaji, V. (2012). Fatal Chromobacterium violaceum septicemia in a South Indian adult. Journal of Infection in Developing Countries, 6(10), 751-755. | 14. Liu, D. X., Didier, P. J., & Plauche, G. B. (2012). Chromobacterium violaceum infections in 13 non-human primates. Journal of Medical Primatology, 41(2), 107-114. doi: 10.1111/j.1600-0684.2011.00529. | 15. Mahmud, Z. H., Paul, D., Akter, S., Kamruzzaman, M., Gope, P. S., Islam, M. S., Endtz, H. P., Cravioto, A., & Islam, M. S. (2009). Characterization of Pigmented Chromobacterium violaceum isolated from Northeastern Hilly Region of Bangladesh. 12th Annual Scientific Conference (ASCON XII), ICDDR, B, Dhaka, Bangladesh, 9-12 February 2009. p. 186 | 16. Manjunath, M. (2007). Fatal Septicaemia due to Chromobacterium violaceum. West Indian Medical Journal, 56 (4), 380 | 17. Narayanan, S., Prasad, T., Nair, I. C., & Jayachandran, K. (2012). A novel exploitable feature of Chromobacterium violaceum: experimental evidence for phenol degradation. Novus International Journal of Biotechnology & Bioscience, 1(3), 1-11 | 18. Orsetti, A. G., Markiewicz, P., Epstein, M. G., Conceicao, O. J. G., D'Ippolito, G., Ribeiro, M. A. F. (2013). Liver abscesses by Chromobacterium violaceum: a case report of a rare disease. OA Case Reports, 2(2), 19. | 19. Rai, R., Karnaker V. K., Shetty, V., & Krishnaprasad, M. S. (2011). Chromobacterium violaceum septicaemia- A case report. Al Ameen Journal of Medical Science, 4 (2), 201–203. | 20. Rashid, Z., Ali, U., Sulong, A., & Rahman, R. (2013). Chromobacterium violaceum infections; a series of case reports in a Malaysian tertiary hospital. The Internet Journal of Infectious Diseases, 11 (1), 1 | 21. Reid, H. D. (1997). An investigation of Pseudomonas fluorescens in the milk environment. Thesis submitted in partial fulfillment of the requirements for the Degree of Masters of Science in Microbiology, University of Canterbury. | 22. Yang, C. H., & Li, Y. H. J. (2011). Chromobacterium violaceum infection: a clinical review of an important but neglected infection. Journal of the Chinese Medical Association, 74 (10), 435-441. doi: 10.1016/j.jcma.2011.08.013. |